THE

CULTIVATION OF ANIMAL VACCINE

AND

EXPERIMENTAL PROOF

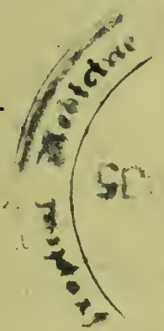
OF ITS

ORIGIN FROM SMALL-POX VIRUS

BY

Surgeon-Major W. G. KING, M.B.

Indian Medical Service, Madras.



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1891.

cultivate the virus
on Potatoes and then
inoculate into calves.

The Cultivation of Animal Vaccine, and Experimental Proof of its origin from Small-pox Virus.*

The determination of the Madras Government to introduce as rapidly as possible a complete system of vaccination involving the use of animal vaccine lymph to the entire exclusion of the humanized form, has given rise to more than usual attention to the subject of methods permitting of its preservation and safe transport. My late post of Acting Inspector of Vaccination gave me an excellent opportunity of carrying on certain experiments on the point, and it is with reference to these that I propose to occupy your attention. I am afraid that the matter may prove of but small interest to a Society that, month after month, has been absorbed in discussing brilliant instances of medical and surgical skill. But, even medical literature has its gooseberry days, and I imagine our Secretary, Dr. Smyth, finding the hot weather fast approaching, suspected that accounts of prodigious tumours might not be forthcoming in sufficient quantity to permit of the issue of the next number of the Journal, and hence asked me to fill the hiatus. If, therefore, I present to your consideration a subject of a more elementary nature than usual, I trust you will understand that your Secretary has medical grounds for not considering it advisable you should be troubled at this hot season with matters not easy of digestion.

In experimentally dealing with a virus, the first step to be taken is certainly to ascertain its origin, composition, and the laws which may affect it in favouring or hindering the ends in view. Seeing that from the days of Jenner downwards, endless pamphlets, articles, and books, have been written on the subject of the origin of vaccine lymph, it is almost a foregone conclusion that the opinions expressed are as various as the authors are numerous. What may be termed the orthodox theory is, that cow-pox is but small-pox exhibited on bovines in a modified form. This fits in admirably with the doctrine of the acknowledged efficacy of vaccination in that the operation endows those subjected to it with a security almost equal to that ensured by the ancient and dangerous operation of direct inoculation, or, when recent, even by an attack

* Read before the South Indian Branch of the British Medical Association, at the April Meeting, 1891.

of natural small-pox. Hence, it is not to be wondered at that constant endeavours have been made to prove in practice, a matter comfortable in theory. Dr. Sonderland in 1830 undertook experiments that, if verified, should have finally put the subject at rest. He maintained that by bringing cows in contact with bedding that had been infected, small-pox had been contracted by them. But, repetition of his experiments in the hands of others has not been followed by results; indeed, were this measure feasible, cow small-pox would, in this country, in consideration of native habits, be an exceedingly common complaint. Jenner relying upon this theory, named cow-pox variola vaccinæ. Of course, a number of his medical brethren consistently opposed this doctrine, as well as the practice of vaccination generally, and, as a sequence, the medical camp has, up to this day, been divided against itself on the subject. Looking at Jenner's work in the light of modern science, I think one cannot help feeling struck with admiration for the man, who, in the midst of prejudices and ludicrous opposition, steadily worked out the details of a branch of medicine, that has been of enormous benefit to the human race. In doing so, doubtless new facts opened themselves before him from time to time, and thus it is, in his writings, there are points permitting of different interpretations, upon which his traducers have gladly seized. The truth is, those who have attempted to prove the correctness of Jenner's statement have certainly not been fortunate in bringing their facts together. In some way or other, accidents have happened during their experiments, or there existed sufficient possibility of such occurring, to afford a handle to those who have entertained opposite opinions. Thus (1830-40) Ceeley's successful experiment of inoculating small-pox after several failures, was subject to the criticism that, as he was avowedly working with both the vaccine and small-pox virus, he may have by some chance changed his instruments, and that therefore his lymph after all was but a vaccine lymph; and, again, on the opposite, that his famous "giant vesicle" really produced benign small-pox virus and nothing else. Chauveau after fifty attempts, declared it impossible to produce anything but papules from which if any lymph-like fluid were taken, small-pox was produced in the human being again. Marson's attempts also failed. Disastrous results followed experiments in Berlin (1847) by the production of fatal small-pox, in Massacheusetts (1836), and in Munich (1839). It is on record also that Cory on five occasions

attempted to get vaccine in this way, without success. Klein also in experiments specially carried out for the Local Government Board, England, declared that successful results could not be obtained. But on the other hand, there have been successful efforts which, although scoffed at, have not been effectually gainsayed. It was reported in 1828 that in Egypt, by whom cultivated is not known, a stock of variolous vaccine lymph in-bred from calves, was secured and is stated to be still in use. Dr. Badcock, of Brighton, produced vaccine lymph after succeeding in 37 out of 200 cases operated upon, and from his lymph many thousands have been vaccinated. More recently, I believe, Dr. Simpson, Health Officer at Calcutta, when in Aberdeen, started a stock, which was for some time in use in the London Animal Vaccine station. This is said to have been now abandoned, as it gave slightly less successful results than the Bordeaux stock, from natural cow-pox there in use. Unfortunately, no details of Dr. Simpson's experiments seem to have been published. The evidence in favour of it being possible to so modify small-pox virus as to render it benign, therefore is just as strong as any that has been produced against it.

It is true that those who held opposite opinions are not without some show of reason, to support their unwillingness to receiving these experiments as unquestioned facts. But I think most reasonable men must admit them to be facts, and not resist this conclusion simply because they cannot *in toto* be explained, on known scientific grounds. Indeed, science of the present day will allow us to accept this theory up to a certain point. We know, for example, the broad fact that certain micro-organisms, which are deadly to one animal are innocuous to others. Thus the bacillus of normal saliva produces septicæmia in rodents, and the bacillus coli of man a like result. The bacillus which—rightly or wrongly—is credited with being the *fons et origo* of typhoid, simply produces septicæmia in the guinea pig. The carnivora can contemplate the deadly anthrax germ with comparative complacency, whilst it proves most deadly to herbivora and man. But, more to our point, anthrax germs cultivated at a certain temperature and then passed through the guinea pig or rabbit are not fatal to the mouse, although still deadly to rabbits and guinea pigs. Again, the virus of hydrophobia waxes or wanes, according as it is passed through the bodies of rabbits or monkeys. But there is a great stumbling block; in all

known instances of diminished virulence of germs, as a result of cultivation under suitable temperature or soil, it is recognized that a repetition of the original conditions will re-produce the former virulence. This being so, as far as analogy is concerned, it should follow that, granting small-pox virus be modified by transmission through the cow, on its return to the human being, or, at least, after one or two removes from the cow, its former virulence should return. But what we are asked to believe is, that a suitable impression by cultivation having been once induced by passage through the cow, this remains for a space of time unlimited—however long the poison be cultivated in its original soil. For example, this must be the foundation of belief in Badcock's vaccine lymph. One way of getting over the difficulty at times suggested is, that the mere fact of removal of lymph for cultivation, always taking place at a certain period of its development since it was implanted in the human being, prevents it reverting to its original state. Those who would accept this theory point to the local and constitutional symptoms produced by taking late lymph, and the presence of rashes of undefined character which take the place of true variola. Granting that the tendency would always be to take lymph at the proper time, it would seem that if there were any truth in this statement, throughout India it should have been possible from time to time since the introduction of vaccination to start an epidemic of small-pox, from the vaccine disease having run riot in the hands of men ignorant of cultivation methods. Thus, those who have any acquaintance with the system pursued by our present class of vaccinators, must know that it is often physically impossible for them to obtain lymph from arm-to-arm in correct time, and yet, by dint of fines and threats of dismissal, they are forced to make a definite out-turn monthly. The facts are well shown in the Sanitary Report for the Punjab for the year 1888, where the Sanitary Commissioner observed :—"Vaccinators, in order to swell their returns, enter every case as successful, whatever be the character of the vesicle, but over and over again have I found, on inspection, numbers of children covered with cutaneous disease, or suffering from diarrhœa, whose arms were one large ulcer as the result of the operations; and it is beyond doubt that spurious vaccination of this kind cannot be relied upon as protective against small-pox, and that small-pox attacks many who have thus been vaccinated is a fact, and fully accounts for the people losing faith in vaccina-

tion, as they are incapable of judging whether the cow-pox is genuine or spurious, or whether the vesicles run a regular or an irregular course." The picture is equally true for this Presidency. The results of this wild system have, however, not been to revert to small-pox, but to bring into existence a lymph that produces local and constitutional symptoms that lack the characteristic of the true vaccine disease, and, *ergo*, we need not doubt, a diminution of protective influence. It is obvious that so far, therefore, no other conclusion can be come to than that if small-pox virus can be modified at all by transmission through the cow, it can be so effectually performed that its original characteristics *cannot be recovered.*

Whether it be in dealing with micro-organisms, or the seeds of flowers or vegetables, or in the breeding of animals, such a statement is in opposition to well-verified facts. But if we contend that small-pox must for ever remain but small-pox, and that even if rendered benign, it must have a tendency to revert, and that vaccine lymph is the product of any one of certain diseases liable to produce vesicular affections on bovines, it would be still possible to advance a theory that would cover the question of protection by vaccination—a theory which I have not yet heard stated, and which, I confess, I should be very sorry to have to hold; *viz.*, granting vaccine lymph now recognized as such, is derived from various and imperfectly known sources, the special micro-organisms of these agree as to producing "immunity," by any of the methods usually suggested with reference to the induction of that state. The theory would lead to the conclusion that there are certain diseases which, once acquired, protect from others—a theory in no way as yet borne out by observations; still, unless it can be shown that the protective influence of vaccination is *nil*, to those who would maintain the dissimilarity of small-pox and cow-pox, there can remain no other reasonable refuge. Much of the confusion which has descended to us must, I think, be traced to Jenner not having at first ascertained definitely that the grease of the horse's heel, which formed the origin of his lymph in later years, was of two varieties—a constitutional and a local; the latter being an eczema or impetigo of the horse, the former the true horse-pox. Seizing upon this, it has been maintained by anti-vaccinators that equine lymph is derived from a venereal disease of the horse; whilst, with apparently good evidence to support it, it has been stated that the origin of a stock of lymph used in Bengal,

was from animals affected with cattle plague. Indeed, notwithstanding the positive evidence quoted, consultation of various text-books of the present day leaves the reader in a "complete fog" as to what is to be believed, and what not. Thus, in the recent edition of Lewis Parkes' work on "Hygiene and Public Health," the following summary is given:—

"The evidence in support of the view that cow-pox is human variola, modified by its occurrence in the cow, is somewhat conflicting, experimental observations on the production of vaccine vesicles in the cow by inoculation with human variolous matter having usually failed. Whatever the original origin of cow-pox in the bovine species, it is certain that the disease is now transmitted directly from animal to animal, and that its origin from human small-pox is an event of very rare occurrence, if it ever happens at all."

From the foregoing remarks, it will be understood that the subject is still surrounded with doubt, and, consequently, merits at the hands of those interested in vaccination, careful enquiry. *In this country the matter is one of peculiar importance,* inasmuch as it is most desirable to prove to natives that the vaccine disease is true small-pox, modified in virulence and deprived of infectiveness by passing through the holy cow, and thus finally to dispose of the constant objection as to the supposed insult of the goddess Mari, by the acceptation of vaccination. Having calves at my disposal during the tenure of my present temporary work, I have thought it advisable to make experiments on this disputed point. As it seemed to me possible that some of you might like to take the opportunity of seeing the results upon animals operated upon up to date, I have directed them to be brought here. At this stage, it would be rash for me to venture an opinion as to what will be the final deduction; all that I can at present say is, that my hope is that a true vaccine lymph will ultimately be produced, but that up to now, all that I have found is that bovines are subject to true small-pox, which can be induced by inoculation. Besides affording you the opportunity of inspecting the calves, I show you pictures of effects obtained up to date, and I have but to add that should any medical officer feel inclined to watch the various experiments made, I shall be most happy to see him at my depôt. It is my intention later on to formulate my experiments with due care;* but in the meantime I may recount, in general language, the results attained up

* See Appendix.

to date. In view of the repeated trials made by other experimenters, I consider I have been most fortunate in being able to record positive effects on my first attempt. Calf No. 1 was inoculated from small-pox lymph of five days' duration. Of the variolous origin of this lymph I have not only satisfied myself by its effects, but also by securing a certificate from the principal residents in the village whence it was obtained, stating that the lymyh was actually taken from a child suffering from small-pox. The child supplying the lymph was also inspected by one of my Superintendents, Mr. Thirumodi Swamy, L.M.S. The calf inoculated with this lymph was a young bull. Both lines and punctures were employed on and near the scrotum. For the first three days, there was every appearance of local results at the points of insertion, as far as the papule stage was concerned. I then noticed on the scrotum, about one-fourth of an inch from an inoculated "line," a fine broad papule arising. My belief is that it was a secondary vesicle, but, as it was close to the site of the original punctures, I was left in doubt; but so far as examination would prove the fact, there was no sign of a puncture being made at the point. Lymph was taken from this papule just on its verging into the vesicular stage, and was used for calf No. 2. In the meantime, a crop of secondary papules appeared on the perineum, where there was not the slightest doubt as to there having been no inoculation effected. Another also appeared on the thigh. From lymph expressed from these, I inoculated calf No. 3. *The papules found at the original site of inoculation of the small-pox virus in calf No. 1, now aborted and completely disappeared*—the secondary eruption, however, continuing its course.

In the first and second calves again distinct secondary eruptions appeared. A transfer to a buffalo of the lymph conveyed by the first—the doubtful secondary vesicle—produced no result, although the crop in calf No. 2 was very favourable. As the buffalo has numerous pitted marks over the body, the cause of failure will be further investigated. *I have thus the lymph from the true secondary vesicles only in operation.* The third calf has now no secondary eruption, and the general appearance is identical with that of true benign vaccine lymph.* Finally calf No. 1, which I would remind you was that inoculated direct with small-pox virus, was inoculated seven days after appearance of the secondary small-pox vesicles,

*A secondary eruption subsequently appeared. See tabular statement.—W. G. K.

with ordinary fresh animal lymph, and the result is *nil*; showing, I think conclusively, that vaccine and small-pox virus are one and the same, and that hence Jenner's contention that vaccinia is but small-pox modified by transmission through the cow, is absolutely correct. I need not say that, warned by results that other experimenters have met with, none of the lymph derived from these calves is at present being issued from my depôt; nor will I take this step till I am completely satisfied that the true characteristics of vaccine lymph have been attained.

Leaving thus the question of the origin of vaccine, there is at least no doubt as to its usual physical characteristics, and the symptoms it produces—facts at least aiding the argument that it is the derivative of but one special poison. A little over twenty years ago, it was believed that vaccine lymph possessed no microscopical characters. Keber however found that it contained solid bodies, and that filtration through porcelain demonstrated that the active portion consisted of this solid—not the liquid constituents. Burdon, Sanderson, and Chauveau confirmed these experiments. A step further, and it was proved by Cohn that these solid bodies were micrococci, and, as a just inference, that upon the existence of these living organisms the activity of the lymph depends. But unfortunately here the matter stops. According to Koch's law, which is accepted as that guiding all work by bacteriologists, before it can be definitely concluded that the organisms suspected are the real cause of a disease, it must be shown that a pure cultivation on suitable media can produce themselves from generation to generation, and on insertion to the body again originate the same disease. Midway the proof of identity of the virus and these organisms has therefore been arrested. As shown by the experiments of Buist, cultivation external to the body on various media is followed by white, yellow and brown products; but it has not yet been possible, under this condition, to cultivate the virus from generation to generation, and thus reproduce the disease. Indeed, the sum of £1,000 is awaiting any one who can, before next month, prove how this can be done—an offer made by the Grocers' Company. Little doubt however can exist as to these micrococci being the active agent of vaccine. Buist claims indeed to have solved the difficulty by actually cultivating vaccine micrococci, and producing protection in at least one case. The knowledge of this fact has reference to the important subject

of cultivation of lymph. As matters connected with vaccination do not often elicit the attention of medical men engaged in the larger branches of our profession, I have thought it well to place on the table illustrations borrowed from Buist as to the microscopical aspect of vaccine lymph. A microscope and lymph are also available for those who wish to examine it. Fig. No. 1 depicts the condition of clear lymph. Fig. No. 2 of opaque lymph. Upon the question of the properties of clear or opaque lymph respectively, depends all that is known, or, at least what forms the chief guide, as to lymph cultivation.

In "clear lymph" are minute micrococci which are supposed by Buist to be spores; in the "opaque" are the full grown microbes. In the former, the micrococci are seen with very little tendency to arrangement, measuring but ·15 micro-millimetre; in the latter, they become streptococci and reach a size of 1 micro-millimetre; torula bodies are also seen in the opaque variety. These are conditions sufficiently marked to render it possible within certain limits to state by microscopical examination, the stage of development of any given specimen. Of course, the fact that lymph has a spore and a fully developed microbe stage is not admitted on all sides; * and, it must be remarked, that it is usually supposed that with bacilli only is this mode of growth followed, whilst to micrococci is assigned fission. But it is possible to meet this objection by stating that by "spores" is meant simply the immature condition of the larger microbe found in opaque lymph. Leaving this microscopical aspect of the question, it is found that in practice the later the lymph, that is, the more opaque it is, the greater are the constitutional and local results. Were it not for this fact, the cultivation of lymph, which is a matter that demands the greatest care and attention, would be a mere rule of thumb.

Nor is this the only subject which must engage attention; all medical men are familiar with the variation of type of vesicle in different human beings, and it is therefore not surprising to find

* It was long taught that good lymph should contain no microscopical form. Klein and Crookshank, however, recognize the vaccine microbe as the "streptococcus vaccinæ"; but the former in describing it recalls the incomplete results of cultivation experiments, and points out that similar micrococci are found in other vesicles produced on the skin. Flugge regards them as "impurities," but nevertheless considers that the action of vaccine depends upon micro-organisms. I have examined a large number of samples of lymph, and have never found the micrococci absent. It seems to me but reasonable (till it can be proved that Buist is wrong) to regard these constantly present microbes as the causative agent of vaccine.

that it is necessary to choose calves with care for lymph transfer. According as the skin of the animal is thin or thick, white or black, whether the lymph used for inoculation has been gathered at the correct hour or not, will the nature and amount of vaccine derivable differ. The temperature of the animal's surroundings, the character of its food, the extent of manipulations it has gone through, its nervous condition and presence of intestinal disturbances also are matters that greatly affect vesicle development. To obtain the lymph in time to prevent the entering on the stage of the fully developed microbe, the cultivator of lymph must be content to devote himself absolutely to the work, and be as ready to take the lymph at 2 o'clock in the morning, as in broad daylight. It must be remembered that this statement is derived from experience of work in a tropical climate. If this be correct, I think it must be evident that a system for the supply of fresh lymph where its cultivation must depend upon the efforts of vaccinators of the stamp now found in this country, is absurd. Indeed, without the aid of men specially educated for the purpose, and possessed of capital sufficient to pick and choose the calves offered, it is hopeless to expect that fresh lymph can be retained in an efficient state of cultivation.

In the matter of cultivation of lymph, all who are acquainted with Dr. Barclay's interesting pamphlet on the subject of animal vaccination, must recognize the importance of attention to the question of "regeneration." In the absence of positive statements of the description given by Dr. Barclay, as a result of his observation of the work of specialists in Germany and elsewhere, I think most medical men would come to the conclusion that the best way to cultivate lymph would be to select the most typical vesicles for transfer, secure them at the correct stage of ripening, and sow the lymph on favourable soil. But in this pamphlet is laid down a system of trusting to human lymph to secure regeneration, as pure animal lymph was supposed to undergo degeneration after a few removes. As a matter of fact, the only authority* I can find on the subject declares that retro-vaccine, which such lymph really is, is subject to rapid degeneration. This system is misleading, and I find does not accord with the present practice either in London or Berlin; in both of which places selection and cultivation are trusted to. In my early attempts however, I was not free from

* Peuch: Journal Veterinaire, February, 1890.

difficulties on this question. I had to deal with a lymph stock which required patient cultivation to render it benign, and my first stock not only had a tendency to run riot, but I was dubious as to whether I could succeed in maintaining it in a fit condition for distribution. Human lymph was therefore imported from time to time; but I soon came to the conclusion that once a good stock was established, there existed no reason why it should be allowed to degenerate, if care were exercised. But did such a result occur by careless treatment, I should think better effects are attainable by the use of animal lymph passed through the donkey than through the human being. In passing through equine animals there is a tendency to increased vigour, and, as Warlomont pointed out, after a series of experiments, this tendency was so marked as to render them unfit media for continued cultivation. The useful suggestion to utilize the donkey, which in this Presidency is the cheapest equine animal for vaccination purposes under emergency, was made by Dr. O'Hara of our Service. It was popularly supposed he advised that these animals should be used in lieu of calves. A perusal of his original communication on the subject, however, shows that he was misunderstood. He simply suggested that they might be used as a source of fresh lymph, when calves could not be procured, and also for the regeneration of lymph. The effects of transmitting lymph through the donkey, where by ill-management a degeneration has occurred, so far as my experience yet goes, are, I think, good. It has the decided merit of causing a lymph that has become accelerated, to be more normal in its development. The reason would seem to be that, irrespective of gain of vigour upon a favourable soil, there is acquired the retarded tendency of donkey lymph. Thus, I find if lymph be taken from a donkey, as early as absolutely necessary in the calf, the supply is scanty, and has not acquired full infective powers. The question of taking lymph at its proper hour is also of importance as to its retention of activity during storage, seeing that under the stimulus of gentle heat, opacity and acidity of the lymph takes place, showing advance from the "spore" stage (if this theory be admitted), and that all the evil results of late stored lymph are thus induced. When dried, however, at a low temperature, the vitality of the spores becomes dormant, and under the influence of gentle heat and moisture they again spring into activity. To this fact, the prolonged vitality of the vaccine and variola scab is due.

I have thought it desirable to enter thus far into the question of the origin and nature of lymph, as essential for the consideration of methods of preservation. Probably, reasoning from modes pursued with regard to small-pox lymph retained for inoculation purposes, the use of charpie for preservation was the first brought into use. Thus, the Brahmins of Northern India preserved their small-pox virus for inoculation from one season to the other, and having made scarifications, introduced it by a pledget tied on the arm of their patients. In a Presidency where my suggestion to use scarifications instead of punctures, has been regarded as novel and, by a conservative few, an alarming innovation, the fact that this mode is indigenous is worthy of note. It was by the use of this material also that vaccine lymph (dispatched by Dr. Carro of Vienna) first reached India. Charpie soaked in lymph was put in a glass having a depression in the centre; oil was placed around the edges, and a covering glass was pressed, so as to exclude air. Finally, layers of wax were rolled round the whole. Lymph thus reached Bagdad safely, and children having been successfully vaccinated, lymph was sent to Bombay. Here one case out of five vaccinated only proved successful, and from this the whole of the humanized lymph for India has been derived. The stock was of equine origin. Animal lymph was first successfully imported by Dr. Blanc of Bombay in 1869. This was derived from Warlomont, who, again, obtained it from a case of natural cow-pox in Naples. I need not remind you that the Brahmin practice illustrates the dry, and Carro's the wet method of preservation, the medium being the same. The most general application of the dry method, is by the use of points. Buist considers this the most favourable procedure, as it arrests the growth of the spores; whereas, preservation by the wet method does not do so. Storage between glasses, or on glass points contained in bottles, is also on the same principle. Wherever the wet method has been pursued, necessarily, to prevent evaporation, air exclusion has formed a chief part of the process. But, reasoning from a bacteriological point of view, it is obvious that the exclusion of air is also essential with reference to the prevention of the further progress of the spores towards maturity, and, the production of an opaque lymph, and its evil consequences. The capillary tube of Dr. Husband affords this protection to a certain extent, and with humanized lymph, in the moderate climate of Europe, has proved of benefit. Müller discovered glycerine

added to vaccine lymph retarded its deterioration, when stored in tubes. This mode gave increased advantages to preservation, together with the greater economy secured by dilution. Yet, I would point out to you, with reference to the stoppage of the growth of the micrococci, that Ruist's cultivating fluid consists of glycerine and an alkali, and that clear lymph is alkaline; thus, rendering it at least possible that cessation of growth of the vaccine spores is not absolutely certain, and, as a just inference, that the use of glycerine is not an advisable medium of preservation.

I think it may be taken as granted that the largely increased use of late of animal vaccine in all parts of the world, is due to the persistent and intelligent advocacy of Dr. Warlomont. This increased use shortly brought a difficulty into observation. It was found that animal vaccine did not, when stored, retain effective powers. This fact is not ascribed so much to the devitalization of the micro-organisms as to the tendency of the lymph to coagulate being greater than in the case of the human product. Finding this to be so, tubes of wider calibre have been employed; but there is no reason to believe that any improvement has thereby been effected. In England, it was found, animal vaccine being usually distributed only to start new stocks of humanized lymph, that the dry method on points sufficed. On the Continent other modes were pursued; the whole vesicle of the animal was cut out, and retained soaked in glycerine. When vaccine was required, a little of the scrapings of the vesicle was removed for the purpose. The vesicle was also dried, and rendered into a powder separated mechanically by sugar lactate. Finally, an emulsion was formed of the well pulped vesicles of the calf in water and glycerine—forming the so-called "glycerine emulsion." This process is that now pursued all over the Continent of Europe. Dr. Warlomont also prepares a mixture, which is known as "Warlomont's Pomade." From its name, it evidently approaches in consistency a preparation which is now in use in the Madras Presidency—"Lanoline-vaccine." What the composition of Warlomont's Pomade is I do not know, and I presume he has preferred to keep the preparation secret. Thus, in his book, the only allusion to its composition made, is as follows:—"Should it be desired to make a pomade of it, we incorporate it with an appropriate a septic excipient, as we now do." As lanoline was not yet known

to the modern world, when Warlomont formed his pomade, it is at any rate certain that this does not enter into its composition. In this Presidency until recently, storage in tubes or between glasses, was the favourite method of distributing animal lymph. Degeneration, however, rapidly occurred, and thus stored, it was found animal lymph became inactive at the end of four or five days; though occasional individual tube contents gave evidence of better duration. Doubtless this absence of vitality under storage, was rendered more marked when judged by "vaccination success" by the system largely pursued in this Presidency of using punctures for the insertion of lymph, whether it be fresh or stored. As an instance of the effect of such storage, I may mention that from the Guntur Depôt lymph for 485 cases was issued, when 22·7 per cent. of failures resulted—although, at the Head Quarters, the rate of success with calf-to-arm lymph was very good. The introduction on an extensive scale of animal lymph to the Presidency therefore presented certain difficulties.

My first object was to decide how long lymph stored by various known methods would survive during the hot weather, and consequently I carried out experiments from March to July in Madras. As to storage of unmixed animal lymph, I find that between glasses and in tubes it could not be relied upon after the first twenty-four hours, and that but small success was obtained up to three days. Non-conductors, such as Glauber's salts, saw-dust, and cotton wool, were employed as surrounding media. Of the three, saw-dust and cotton wool ran each other closely, as to efficacy. But, I think, saw-dust, on the whole, preferable; yet protection by these failed to effect what was required. It thus was incumbent upon me to ascertain some more reliable system. In setting out upon this task, I made it a *sine quâ non* that the mode employed should be absolutely simple, and that the use of any elaborate apparatus for manufacture, storage, or transmission should be avoided. A matter of importance was obviously to obtain a combination that should not be affected by heat during transport. The question of the preservation of fluid lymph, was naturally first undertaken. Exclusion of air by oil is utilized for the preservation of certain wines with success. Adopting this idea, I therefore attempted the making of seals with gingelly oil, at two ends of tubes containing lymph. Only in one instance was any result obtained up to six

days, and here transport was by rail only; where a road journey was involved, failure resulted.*

With the glycerine seals the same results followed. I then mixed lymph with glycerine; upon this my notes were as follows:—

"In Europe, the preservative universally used is glycerine. This has been used in temperate climates successfully to a dilution of ten parts; but it was thought advisable to limit the question as far as possible to duration of efficacy, instead of complicating it with the effects of excessive dilution. Proportions from 1 to 3 were therefore not exceeded. Two specimens were collected on the 3rd, six on the 5th, and three on the 8th March. None of these specimens gave successful results after seven days' storage in Glauber's salts.

Conclusion.—When subjected to conditions of a tropical climate, plain glycerine is not sufficiently preservative to cause retention of efficacy in animal lymph with which it is mixed, for seven days; although within that period the compound survives better than unmixed lymph."

I now considered whether it might not be possible to obtain among the mild forms of antiseptics, some substance more suitable than glycerine. Boro-glyceride was the first that suggested itself to me, and I may add that the same idea struck the Chief Secretary to Government, Mr. Price; as, shortly after my experiments had been conducted, of which at the time he was ignorant, I received a communication from him making the suggestion. The effect of boro-glyceride was so encouraging, that I thought I had at last hit upon what was required. Activity although in a diminished form was obtained up to 15 days after storage—a very great advance upon results hitherto obtained—otherwise. Another antiseptic that seemed likely to prove useful was Lister's new product, the double cyanide of zinc and mercury. Lister claims for it, that it is distinctly antiseptic in action, and devoid of germicidal properties; so that he deems it necessary to combine with it, the use of perchloride

* I may here remark that a vast difference may be expected in this country in the results of such experiments, as to whether the mode of transport be by road or rail. Subsequent experiments conducted by me as to protection of lymph from heat, show that through a thickness of half an inch of teak and half an inch of saw-dust, a temperature of 112° and more is obtained by an hour's exposure to an ordinary midday sun, and, consequently, that when in a climate where *in vacuo* the sun's rays often afford a temperature of over 150°, great care as to transport is required, as vaccine perishes absolutely at 140°. In short, one of the greatest difficulties to be surmounted is to ensure protection by suitable apparatus from heat during the exposure, which packages must undergo during transmission to vaccinators, or during the time they work with it in villages.

of mercury in the new gauze dressing. This then answers the description of the desired preservative almost typically. At the time of making my experiments, I could obtain no double cyanide in the country except in the form of gauze. This I procured, and following the directions as to washing out the perchloride, I hoped to obtain satisfactory results. But, though the gauze was simply saturated with vaccine lymph and pulped matter no success followed, proving that the trace of perchloride left in the gauze had effectually killed the vaccine micro-organisms. Luckily, the double cyanide although practically insoluble in water is soluble in glycerine; thus giving further hope of its being a useful substance with which to attempt combination. Solutions of various strengths were used, and gave success up to thirteen days. Saccharine, which possesses antiseptic properties, was also employed by me, in various strengths. The result here was much the same as with other mild antiseptics, activity was ensured up to 17 days. But it was evident that however mild the antiseptic employed was, and however consequently feeble its action upon pathogenic organisms might be, in the end, deterioration of activity was exhibited. I nevertheless think the results by all these methods were exceedingly satisfactory, when compared with former recognized modes of treating animal lymph.

I then turned my attention to the use of vesicle pulp with glycerine, which, as I have already remarked, is chiefly relied upon in the Continent of Europe. The results with this combination are thus stated in my notes: "*Conclusion.*—These preparations, which are those chiefly employed in European practice, are not trustworthy in this country, after from the 9th to the 12th day during the hot weather." This conclusion has since been questioned by a Member of the Leprosy Commission, which lately called at Madras. This gentleman very kindly placed me in communication with Dr. Doering of Berlin, the Director of the Vaccine Supply Institute there. In a letter on this subject, Dr. Doering suggests I must have made a mistake, and probably that this consisted in my not using glycerine diluted with water. It is of course quite possible I may have made not one, but a dozen mistakes; as, after practising the cultivation of animal lymph for several months, I am by no means convinced of the simplicity of the processes involved. In fact, the man who cultivates calf lymph successfully is not born, he must be made, and in the making of him not a few failures must be

expected ! But having been kindly provided by Dr. Doering with a specimen of his lymph preserved by the glycerine method, I have had an opportunity of observing its results, and now have the pleasure of exhibiting it to you. My experiments with it are not completed, but I may state at once that there can be no difference of opinion as to the results obtained by me, when compared with those by Dr. Doering. This is not due to its mode of preparation, but its after treatment. The glycerine paste in Germany is placed in tubes, containing just enough for five cases each. It is prepared in a climate, be it remembered very different from that of Madras in the hot season, and not only so but storage over ice is maintained. The tubes sent to me are hermetically sealed, and, as you see are very ingeniously protected from heat, by the use of two tubes of solid wood with a surrounding layer of fine sand. This however was not the description of test to which I put the compound, made by me in Madras at a temperature in the shade of over 100°. It was distributed in tubes having the corks frequently subject to removal in the process of effecting vaccination, and packed in a tube filled with saw-dust as a non-conductor or in an open tin box. I had no reason for prejudice in favour of one substance more than another. My search was for a combination that would serve the requirements of vaccinators, under conditions obtaining in this Presidency. Hence, the method of preparation and careful storage was by no means the only question to be considered; the main point was to obtain a preparation that would stand the test of being exposed to air, and a high temperature. Thus tested, I found that the glycerine preparations were untrustworthy. I show you glycerine preparations made on the 25th of last month, and the odour of each will readily prove to you how vast a difference results from the method of storage and the consequent degree of exposure to air—here illustrated by the use of a wide and a narrow tube respectively. Whilst, therefore, glycerine preparations may not be affected by this climate when hermetically sealed, and care is taken as to their exposure to high temperature they certainly cannot stand free air contact; a fact, I think, which favours the opinion that, as shown by Ruist, glycerine permits of cultivation proceeding. In this estimate of the untrustworthiness of glycerine preparations, I have since found that I am supported by experiments made in Bombay and Rangoon. Although it would be

Ruist

premature to pronounce a definite opinion, I believe as to lasting powers under conditions found in this climate, there is no likelihood that I have erred, in advising rejection of the German method for the purpose of the Vaccination Department of this Presidency.* With the pulp I tried, as I had with the pure lymph, a mixture of boro-glyceride; again without successful results. I ascertained that no hard and fast line can be drawn between an antiseptic and a germicide so called, and that consequently prolonged contact, even where the activity is weak, ultimately causes degeneration and death of the vaccine micrococci.

I had, of course, during these experiments, kept my attention considerably upon matters referring to vaccine lymph. In reading an old number of either the "British Medical Journal," or the "Lancet," I stumbled across a case of anto-inoculation. The patient had vaccinated his own nose, after rubbing some vaseline upon his arm, which was giving him trouble from itching. The fact that the patient had been able to convey the vaccine virus to his nose, notwithstanding its impregnation with vaseline, made me come to the conclusion that vaccine micro-organisms must be capable of remaining uninjured in contact with vaseline. If this were possible with vaseline, where from its careless preparation free acids must be frequent, it seemed to me a matter of surety that vaccine would remain in contact with Liebriech's lanoline with impunity. Its ready absorption by the skin, its bland character, and its resistance to decomposition, showed that it would offer peculiar facilities. Gottsein had proved that, "(1) The bacteria which effect a spontaneous decomposition of glycerine fats belong presumably to the class of anærobes; a number of ærobe germs (even the putrefactive) perish on a medium containing fat. But the term of continuance of this retrogressive metamorphosis is decided by the proportion of fat to the other ingredients of the nutritive medium; (2) Free fat contains anærobes for some days after it is exposed, but lanoline has under similar circumstances neither ærobe or anærobe germs; (3) Glycerine fats may be so impregnated with bacteria that the latter can pass through the fat to the lower-lying infectible substances, while

* My experiments are now completed, and have afforded me good reasons for regarding the conclusion originally arrived at by me as to the unsuitability of glycerine preparations, to be absolutely correct.

lanoline cannot be permeated by bacteria. It acts therefore as a preventive of decomposition when laid over infectible substances." I therefore on the same date made a preparation of pulped vesicles and lanoline, and pulped vesicles and vaseline. My inference that vaseline did not destroy the vaccine proved correct, but the amalgamation with vaseline was less perfect than with lanoline, and the uniformity of success was consequently proportionately smaller. At one stroke, results were obtained showing that the duration of animal lymph preservation, which up till now managed by other preparations was about fifteen days, could be at least doubled. Professor Liebriech, whose scientific work brought lanoline into notice in its present pure form, has very kindly expressed an interest in the use I have put it to, and in a late communication he informed me that he had forwarded the results I had obtained to Professor Gottsein; from him, I expect further information as to the action of glycerine and cholestrin fats when exposed to air.

As might be expected possibly from less even mechanical distribution, and probably from less concentration of vaccine organisms than when prepared in the form of pulp, *plain lymph* mixed with lanoline or vaseline did not give satisfactory results. With lanoline and vesicle pulp, I prepared combinations of various strengths, and came to the conclusion that 1 in 7 made a safe compound. Experiments were made up to a dilution of 1 in 10, and good results were obtained. A combination was made with glycerine and lanoline, but the result proved that lanoline derived no advantage from the admixture, and again forced upon me the conviction that when exposure to air is contemplated lanoline is a superior substance to glycerine. This lanoline preparation was proved to stand transport to all parts of the Presidency, and gave successful results up to forty days. I consequently advised that this preparation should be employed by Government. It seems to me that it especially suited the requirements of vaccination in this Presidency in that the mode of protection was simple, and that any amount of vaccine could be withdrawn as desired, without it being anticipated that, as a result of exposure, the rest of the vaccine would decompose. Since this period, further experiments have proved that its lasting powers are longer than I originally thought. Thus a specimen which I took with me to various districts on tour gave the following results:—On the 27th day a calf yielded a splendid crop from inoculation; on the 41st day two children

were successfully vaccinated; on the 61st, 71st, 101st and 106th* days successful inoculation of calves was accomplished.†

In advising the adoption of this system, I informed Government that "so advantageous is the use of the *vesicle pulp* as compared with *extracted lymph*, that the paste will, in the practice of skilled men, serve for the purpose of successful vaccination better than freshly-stored lymph." Further experience of the paste, however, shows that it is quite equal to fresh lymph in efficacy, as judged by "insertion success" and "vaccination success." The possibility of preserved lymph being able to compare in efficacy with fresh lymph has seemed to some paradoxical; but, in reality, the explanation is exceedingly simple. In squeezing lymph from the calf to satisfy requirements in the "fresh lymph" system, only a certain number of the total micro-organisms a vesicle contains can be dislodged from the interior; for it must be remembered that the anatomical structure of the vesicle of the calf differs from that found in the human being. But any method of preservation that necessitates dealing with the complete vesicle, places at disposal the whole of the contained organisms. It therefore becomes simply a question of the extent of dilution, as to whether bulk for bulk preserved vesicle pulp shall be as effective, or even more effective than fresh lymph. In actual practice it could hardly be expected that with the uneducated class of vaccinators up till now employed in this Presidency, anything approaching uniformity of results could be expected, seeing that individual skill in operating must necessarily differ within wide ranges; nor, on the other hand, could it be hoped that my first efforts would be free of errors of judgment; thus, of 22,040 cases reported and verified up to the 15th February, I find it desirable that in making the calculations of percentage of success, 3,891 should be excluded. In saying this, it must be remembered that I have not excluded individual cases, but the whole groups referred to. These embrace one group reported from the Chingleput district, where the percentage of success reached only 80·61. The paste

* It gave successful results up to the 126th day when the sample was exhausted. A portion of another paste that was sent to Dr. Waddell, Deputy Sanitary Commissioner, Darjeeling, having been returned to me by that officer, gave good results on a calf at the age of 137 days in my hands.

† Another portion of the same sample was given to a Member of the Leprosy Commission—Dr. Thompson—which he took with him to Burmah and Northern India. He reports that of twelve insertions on children, two were successful (on different individuals) and 25 out of 31 insertions on the calf, 130 days after preparation. Lymph from this cultivation on the calf was employed for the vaccination of six primary cases, and twelve re-vaccinations; of the former, three were successful, and in the latter, the whole were successful.

In his official letter, No. 396, of the 12th July, the same officer writes as follows:—"On the June 11th, I successfully inoculated a calf with the remaining portion of paste, and from the animal a number of children were vaccinated. *The paste was then 6 months old.*" [Italics not in the original.—W. G. K.]

was experimental in nature, and the District Medical Officer was duly warned of the fact before issue. A second number was reported from the Trichinopoly district where, by mistake, paste had been forwarded to men who had never been trained as to the mode of application; here but 82·61 per cent. was attained. Again, to the South Arcot district a paste was unluckily issued which had been made from inferior vesicles, the finest, which had been first taken, having been kicked from the pan during the process of stripping; here but 77·90 per cent. was produced. The number rejected also includes cases performed by individuals who had asked for paste, and to whom no demonstration of the method had been made. If the whole of these bad groups be included, the percentage of vaccination success in 22,040 cases is 92·33 per cent.; but if excluded, it amounts to 94·67 per cent. in 18,149 cases. Judged by the percentage obtained in certain other parts of India with fresh animal lymph, this result does not appear brilliant. Any comparisons made by me, however, must necessarily deal with those obtained by the Vaccination Staff of this Presidency under changed conditions. *Thus when compared with the average percentage of* 93·2 *gained with fresh animal lymph in this Presidency during* 1890-91, it is seen that there remains 1·47 per cent. in favour of the animal lymph preserved by the lanoline process, and that even taking the unfavourable groups into account, the difference in favour of fresh lymph is only ·87 per cent. The average percentage upon 13,371 cases reported up to the 25th March amounts to 95·24—no exclusion of cases being made; there is thus shown a distinct improvement in the second quarter as to results obtained, which is undoubtedly due simply to vaccinators becoming more accustomed to the new mode pursued. There is consequently every reason to think that further practice will produce still more satisfactory results. As it stands, therefore, in the second quarter, lanoline-vaccine shows better results than the average for the whole Presidency for fresh animal lymph by 2·04 per cent. This gain by lanoline-vaccine is rendered more marked when it is borne in mind that vaccination with fresh animal lymph has hitherto been entrusted to either specially skilled vaccinators, or to the Deputy Inspectors only; whereas "lanoline-vaccine" has been issued to the full staff of districts indiscriminately, after but a single lesson as to the mode of insertion. The accompanying table will give at a glance the influence on "vaccination success" of various methods.

Comparative Statement showing Vaccination Results with various forms of Vaccine and Lanoline-Vaccine.

No. of Cases.	Nature of Vaccine.	Name of Operator.	No. of Insertions.	Results per 100 Cases—Insertion Success.								Vaccination. Success per cent. of Cases operated upon	Remarks.
				Failure.	One Vesicle.	Two Vesicles.	Three Vesicles.	Four Vesicles.	Five Vesicles.	Six Vesicles.	Insertion. Success per cent of Total Insertions.		
	ANIMAL LYMPH.												
5,685	**Fresh.** Calf to arm. ..	Dr. Cory, Vaccine Supply Dept., England	5	·39	·16	·47	1·37	4 38	89·87	...	96·25	99·60	
101,153	Ditto ...	Madras Vaccination Dept.	6	...	...	...	...	...	...	...	...	93·2	Average for the year 1889-90.
18,149	**Preserved.** Lanoline-vaccine.	Ditto ...	4	...	...	...	...	...	...	...	...	94·67	Result on first issue of lanoline-vaccine for 3 months.
13,371	Ditto ...	Ditto ...	4	...	...	...	...	...	...	...	...	95·24	Results reported in the 2nd quarter up to the 40th day.
314	Ditto ...	Chingleput Vaccination Staff.	4	...	...	Not available.			...	...	95·69	99·36	Inspected by the District Medl. Officer, Chingleput.
108	Ditto ...	Ditto ...	4	·92	1·85	7·40	19·44	70·37	...	...	89·12	99·07	Inspected by myself.
174	Ditto ...	Trichinopoly Vaccination Staff under supervision.	4	1·13	2·26	2·26	10·73	83·61	...	...	93·36	98·93	Inspected by the Medical Supt., Vaccine Supply Depôt, and the Dy. Inspr. of Vaccination, Trichinopoly.
46	**Fresh.** Calf to arm....	Bellary Municipality Vaccination Staff.	6	2·2	4·3	13·04	4·3	28·3	4·3	43·5	73·19	97·83	Stock of lymph good.
120	Ditto ...	Salem Vaccination Staff..	6	15·8	21·7	9·2	18·3	16·7	5·8	12·5	44·3	84·67	Stock of lymph inferior.
	HUMANIZED LYMPH.												
424,003	**Fresh.** Arm to arm ...	Madras Vaccination Dept.	6	...	...	...	...	...	...	...	...	96·8	Average for the year 1889-90.
55	**Preserved.** In tubes..	Dr. Pearse, Vaccine Supply Dept., England.	6	5·45	12·78	9·1	7·25	14·5	14·5	36·3	66·97	94·55	
267,577	**Preserved.** 1. In Tubes ... 2. ,, Plates of glass... 3. ,, Bottles ... 4. ,, Prepared from scabs ...	Madras Vaccination Department.	6	...	...	Not available.			...	...	...	91·5	Average for the year 1889-90.

In short, the efficacy and trustworthiness of the lanoline preparation are beyond question. Comparisons made as to results with the same paste in the practice of numbers of vaccinators show clearly that inferior results are obtainable only by careless and unskilled men. Of course, if a vaccinator systematically exposes the paste to dust and filth, uses a dirty paste measure, or none at all, or exposes the paste to the direct action of the sun, failure must be expected; but if the simple precautions necessary as to keeping the paste cool and clean be followed, and the insertion be made with common skill, the paste will undoubtedly prove as effectual and trustworthy as fresh animal lymph.

Necessarily, in connection with preservation also arises the question of economy. Calf for calf more being obtainable by taking the whole of the vesicle contents instead of a part, any system involving the use of vesicle pulp must be, *ceteris paribus*, cheaper than the collection of lymph in the calf-to-arm method. Thus calves used by me during the first quarter gave on average in one depôt enough for 770 cases; whilst, at a maximum, enough from a single calf for from 1,700 cases to 2,500 according to dilution practised could at times be obtained against an average of 100, and a maximum of 300 under favourable circumstances of assembly of patients in the fresh lymph system. Both these amounts compare very poorly with the results obtained on the Continent of Europe. Thus, in Berlin, Dr. Doering tells me he obtains 20 grammes of vesicle pulp from a calf, which will yield sufficient for 6,000 persons. I was told by a visitor at my depôt that my vesicles were not so large as those found in Germany, and that this would account for the less yield. I have, however, now had the advantage of seeing vesicles made by the German stock both in the child and in the calf, and I am distinctly of opinion that in no way can the stock brought to its present state of cultivation in my depôt, be considered inferior to that of German origin; in fact, the opposite is my opinion. In the child the areola was decidedly not less than that produced by the Madras product, and in the calf the vesicles were in no way superior, either as to size or amount of lymph; although every possible advantage of devoting good calves for the purpose was offered. In short, the reason why as much pulp cannot be obtained in this country as in Europe, depends simply upon the breed and condition of the calves

employed. Indeed, so fragile as an effect of bad breeding and feeding are our calves that it is impossible to cultivate lymph by "lines" with safety to the animal, at the age generally used in Europe. Greater age must be allowed to secure the necessary stamina; and with greater age the unfortunate complication of a thicker skin must be anticipated. Then, as to area of abdomen which is, of course, of the first importance (for necessarily a large crop cannot be cultivated without a large acreage), I would ask those whose memories of Home have not faded in the midst of the "glories of the East," to recall the size of an English calf six months old when compared to one of this country! Indeed, were it possible to obtain the same amount of pulp from a country calf as from a European, the lanoline-vaccine method would be more economical than the glycerine, as it can be safely diluted up to 1 in 10 which would at 20 grammes per animal produce enough vaccine for 7,202 cases—just 1,202 more than possible, or at least practised, with the glycerine method; and, as I have no doubt dilution can be much further used safely, it must be possible to effect even greater economy.

As already stated, of late years in this Presidency, the method of lymph insertion for vaccination of the human being has been by means of punctures, although here and there modified scarifications have been employed. The method of puncture certainly is not that advisable in dealing with lymph that is preserved, and diluted in the interests of economy: here large surfaces are requisite, and are constantly used. Indeed, where but the average skill obtainable under a public system of vaccination can be reckoned upon, even with fresh lymph—that is, with lymph where the supposed spores are still in full vigour so that a full effect shall be secured, it is advisable that scarifications should be adopted. A very little practical experience of the work of vaccinators of this Presidency, showed me that it was out of the question to trust to their using the ordinary lancet for scarifications. I found the usual result of their clumsy efforts with this the best of vaccination instruments, to be that a certain amount of blood was drawn by making perhaps a single scratch in itself too deep, and that this was plastered over the surface, presenting a horrid sight to the parents and a grim satisfaction to the vaccinator, who incontinently concluded that he had done his duty. This splattering

of blood, however, being wiped away, soon showed that the scratches formed were, often at the cost of much pain to the child, irregular and unsatisfactory. It therefore seemed to me, if this system was to be introduced by Government, that, pending reorganization of the Department (which is very urgently required and which Government has promised to effect), it would be essential to supply an instrument that should not demand of the vaccinator more skill than might be expected of one of his class. I hence adopted a modification of Dr. Weir's "rake." I may state that the arrangement by which the teeth of the rake are protruded in this instrument now shown, permitting of graduation, was designed with the object of meeting the absurd objections which I had reason to believe would be pronounced as to the possibility of vaccinators exceeding the required depth of scarification. Practical examination of the work by vaccinators in various districts, showed me at once that this safeguard was not requisite. I have therefore designed a cheaper instrument without the screw arrangement, which is at present being issued. As soon as the vaccinators show they are to be trusted with instruments affecting smaller areas, these can be prepared, and when the Vaccination Department possesses properly educated men, the plain lancet will doubtless be all that is required; as in reality it does not matter in the slightest degree by means of what instrument the scratches are made, so long as the paste is induced to enter the true skin. Thus I have used successfully the paste with single lines, double and treble lines, and with ordinary punctures, taking care that the paste reaches the *rete Malphigii*. You will see amongst the children present, vaccination marks in the process of "taking" that have been made by punctures by the lancet, as well as by two and four lines. I need not tell you that to reach the true skin, it is by no means necessary to draw blood, and consequently I believe when using the instrument of the nature you see, the operation is both quick and painless, and in this opinion I am glad to say I am supported by several Medical officers in and out of this Presidency. I also exhibit on the table the scarificators in use in the Berars, and in the North-West Provinces, by the Vaccination Departments there, and other commonly used instruments in vaccination. I consider Dr. Little's instrument which is that used in the Berars, which is also a modification of

Dr. Weir's "rake," to be a cheap and ingenious adaptation of this principle.

Further remarks on the experiments, and generally on subjects referred to, in the above paper.

From the above notes, it will be seen that the lymph taken from the vesicle on the scrotum of calf No. 1, concerning which there was a certain amount of doubt as to whether it was truly of a secondary nature or not, was lost after transfer to calf No. 2 by attempting the inoculation of a buffalo. This animal was selected by me simply from motives of economy. The cause of failure was not ascertained. The animal certainly had numerous pitted marks on the thighs and abdomen, that looked suspiciously like what might be imagined to have been caused by a disease similar in its nature to small-pox; but no conclusion could be arrived at. It is very unlikely the animal had been previously vaccinated. Fresh vaccine lymph also failed on the same animal. It is, however, no matter of regret that I lost the lymph from this doubtful origin. I had taken lymph for the inoculation of calf No. 3 from vesicles that were undoubtedly secondary, and from this source the stock has been continued uninterruptedly up to the present time.

In ascertaining whether the small-pox virus would lose its character and assume that of animal vaccine, my attention was chiefly directed to the physical appearance of the vesicles produced, the permanent absence of secondary eruptions and the stages and time of development, as compared with ordinary animal vaccine. Thus regarded, it will be seen that the calf originally inoculated with small-pox virus, was irregular in progress. In the first place, instead of the inoculated parts running a normal course, they followed this only up to a certain point and then faded away entirely, leaving no record of their existence but the constitutional affection of the animal, as evinced by the production of secondary vesicles. Although I grant the single experiment does not warrant my speaking positively, I believe it is with reference to this peculiarity of the action of small-pox virus, that mistakes have been made by the numerous observers who have entered into this subject. When at the site of inoculation the papular elevations have refused to advance further, it has been concluded that the case was a failure, and possibly search was not made for secondary eruptions; indeed,

even where these have been found, I have not ascertained that they have been looked to as the source whence to derive lymph. It seems to me that the observance of this procedure avoids the danger of propagating small-pox by unchanged material squeezed from these sites, as has actually occurred when these have been looked to as sources of lymph. In short, I think I owe my good fortune in being able to obtain positive results from a first experiment, where others have denied the possibility of any being found, firstly to the fact that the small-pox lymph used was perfectly clear—being of the fifth day only—and, secondly, to the accident that the secondary vesicles were rendered visible, by appearing on the belly of the animal which had been previously shaved throughout. In the first transfer then of this lymph from the doubtful secondary vesicle, it will be seen that the development was in exact accordance with the time required in the case of ordinary animal vaccine, except that the scabs fell early, and that secondary eruptions again appeared. But, in calf No. 3, although the general characters of vaccine were entertained, there was more tendency to quick change, and here also there were secondary eruptions. Quick change also occurred in calf No. 4, with secondary eruptions; but, in calf No. 5, the true course as regards time of development was assumed, and it is noted that but a single small secondary vesicle appeared. With the exception then of this single example of a secondary eruption, which even in certain unfavourable conditions of normal lymph occurs from time to time, I considered that by the four successive transmissions of the virus through the calf, the true character of animal vaccine had been assumed. From this animal, therefore, vesicle pulp having been taken, I forwarded a specimen of paste to Assistant Surgeon Gonsalvez, Acting District Medical and Sanitary Officer, Anantapur. I did not tell him the origin of the lymph, but described it "as special paste," and desired him to test it upon two healthy individuals, and let me know the result. He was particularly warned not to use instruments of which there could be a suspicion as to their bearing traces of any other lymph. He was also asked to watch carefully the constitutional and local symptoms produced, and to record them fully. This he has had the kindness to do in a very satisfactory manner, and I have the pleasure of appending his report. From this, it will be seen that the symptoms exhibited were strictly those ascribable to the use of

a good form of vaccine. It may be thought that I played Mr. Gonsalvez rather a shabby trick in sending him paste to try, which I had not used myself on the human being, and which might, according to some, have produced small-pox in the subjects vaccinated. Personally, however, I had full faith in the safety of the experiment, as the symptoms on the calf were absolutely characteristic, whilst I conceived that it was most desirable that a perfectly unprejudiced person should make the experiment and report thereon. Further, and here perhaps is the "shabby" part of the matter, I came to the conclusion it was most desirable—my responsibility in any case being indubitable—that if small-pox were started by the lymph, it should occur in Anantapur and not in Madras! The storm of indignation that doubtless would have arisen in Anantapur might have been weathered, but to have started an epidemic by this means in Madras Town, though there was plenty of the disease about at the time, would have raised a storm that would have finally wrecked my little department. Side by side with the thanks due to Mr. Gonsalvez for his accurate report, I also therefore offer him my apologies.

Having received from Mr. Gonsalvez a telegraphic statement as to the success of the lymph supplied, I caused another stock of lanoline-vaccine to be prepared from calf No. 7, and with this I personally vaccinated several cases at Ootacamund. I now let my stock of ordinary animal lymph die out, *and have substituted entirely the small-pox lymph modified by transmission as described.* I am now issuing from this stock lanoline-vaccine for about 3,000 cases daily, and have not heard of a single untoward result therefrom. On the human being and on the calf, the vesicles are typical in their course and character, and, moreover, are very fine and highly satisfactory as to size.

As a sequence of the observations thus far continued, I no longer trouble myself as to questions of "regeneration"—a matter which, from the conflicting theories which exist, for a considerable time after commencement of my duty served me as a nightmare. I am convinced that in careful cultivation and selection of the vesicles for re-transmission lies the whole secret of successful propagation of vaccine lymph, and I no longer look to either the human being or the donkey for help in this matter. Indeed, if "regeneration" were ever required, I should search for more small-pox virus, and

start a fresh stock. Seeing that not only in my own hands but in those of others, attenuation of small-pox virus has been shown to be possible, it seems to me also absolutely idle that search should be made for it second-hand as natural cow-pox, and that on a solitary case being discovered, a rush should be made to obtain the virus. Further, I cannot see how it can for one moment be doubted that the long opposed theory of Jenner that vaccine is but small-pox virus modified by transmission through bovines, is absolutely correct; and I say this not only with the confidence resulting from seeing that whilst I had sowed small-pox virus I had reaped vaccine (being confronted with a disease corresponding in all respects to that well known to me as that produced by what is ordinarily understood as animal vaccine) but also because the calves, through which the first transmissions of the small-pox virus had been made, proved afterwards absolutely immune to fresh animal lymph of my ordinary stock, which, although it had on a few occasions been cultivated in alliance with humanized lymph, was in parentage of the Naples stock of natural cow-pox (Warlomont's stock).

Another subject illustrated by these experiments is the influence of late lymph on the system. The importance of seeing that clear lymph (generally shown about the 96th hour from the calf) only is used for transfer of lymph, is well explained by Buist. But, in dealing with the transmission of small-pox virus, another question arises. Granting that the lymph so obtained is only modified or benign small-pox and that vaccine is some other undetermined modified product of the cow, if it were allowed to run riot it should tend to revert to its former character. Holding this in memory and partly from a desire to have two strings to my bow, in case any accident happened to the vaccinifer, I used opaque lymph from calf No. 4 for calf No. 6 (see notes). As a result, there was found no tendency to revert to the production of secondary eruptions or to failure in producing the characteristics of animal vaccine; but from the notes, it will be seen that local inflammatory action was very high. I think taking into account facts bearing on this point already noted in my paper read before the Association, this not only proves the moral laid down by Buist, but also demonstrates another caution not generally recognized by the profession, namely, the advisability of relieving the vaccine vesicle in the human being by puncture. Not only in calf No. 6 but also in No. 5, it was

observed that where the vesicle had been removed *in toto* for the preparation of lanoline-vaccine, there was practically no inflammatory action round the site, whereas when the lymph was left to run its normal course—especially in No. 6, where opaque lymph was used—inflammation was severe. I therefore think that to allow the lymph to drain off by puncture, just as the vesicle becomes tense with lymph, is an advisable proceeding, with the object of preventing useless secondary local and constitutional symptoms.

Lastly, I would call attention to the suggestion, conveyed in the foregoing paper, as to the change which occurs in the attenuation of small-pox virus, by passing through bovines being of so marked a nature, *that it is impossible for it ever to recover its former characteristics.* By neglect of the rules of cultivation, I am certain all that can happen to the virus at present forming my stock, is either a progress towards so mild a type as to secure a mere local and varied action on the human subject, or a tendency on the calf, to irregularity and ultimate fading out, as a local inflammatory and pustular eruption. But, so far as our knowledge at present extends, by no manner of treatment can it be made to resume the nature of small-pox virus. This I think most will admit without argument; this being so, it is obvious that, as a result, there must exist for the bacteriologist and pathologist wide fields for study, that may render Jenner's famous discovery of ever-advancing importance to the human race.

Statement showing successive grades of modification of small-pox virus undergone in becoming "vaccine."

Date.	Hour.	Temperature of animals.	Papule.	Vesicle.	Scab.	Secondary eruption.	Remarks.
				CALF No. I.			
19th March 1891.	..	101·2	.....	.....	.....	.....	This was inoculated with 5th day small-pox virus, supplied by 1st class Vaccinator, Baboo Naidu of Trivellore.
23rd March 1891.	77 hours.	102·2	Faint blush and raising of skin. Two punctures and five lines out of seven had a flush of redness and slight elevation, indicating that they were taking.				
			Flat slightly raised oblong papule is noticed at the base of scrotum. This was assumed to be from a puncture; but there is no sign of this having been made.	.....	.....	The papule on the scrotum is probably secondary.	
24th March 1891.	115	99·2	.....	The papule found at the base of the scrotum is vesicular.	.....	A papule is found in the perineum.	*Drawing made.* From the vesicle, calf No. II was inoculated-
25th March 1891.	139	...	.....	.....	.....	Six hard papules are found, one on the right thigh and three close to the one previously noticed on the perineum.	*Lines of original inoculation and punctures have faded away.*

On 7th day Six papules

On 8th day. Vesicles
on 9th day umbilication complete

Statement showing successive grades of modification of small-pox virus undergone in becoming "vaccine."—contd.

Date.	Hour.	Temperature of animals.	Papule.	Vesicle.	Scab.	Secondary eruption.	Remarks.
26th March 1891.	163	101·2	.....	.....	.....	Six characteristic vesicles; four in a group, on the perineum and one on the right thigh having characteristic umbilications. Lymph was found oozing from some of the vesicles.	Drawing made. Dr. Smyth, Resident Surgeon, General Hospital, visited the Depôt and saw the calf. Calf No. III was inoculated from the secondary vesicles on the perineum; one was left undisturbed for further observation. Several glandular enlargements were found in the lumbar region.
27th March 1891.	187	103·6	.....	.....	.....	Umbilication is very distinct.	
28th March 1891.	211	102·6 102·4	.....	.....	.....	Umbilication has disappeared. Scabbing has set in.	
29th March 1891.	235	101·4 101·6	.....	.....	.....	The single undisturbed vesicle, retained to watch progress, has a firm scab—having the ordinary appearance of small-pox scab.	All lines and punctures of inoculation are now free from redness.
4th April 1891.	...	...	.....	.....	Uninjured scabs fell; but may have been detached when exhibiting the calf at British Medical Association on the evening of the 3rd April.		

Note.—This animal was inoculated unsuccessfully with fresh ordinary animal vaccine lymph on the 30th March 1891. This was repeated with a like result on the 17th April 1891.

Variola Vaccinæ.

Calf No. 1 inoculated with small-pox virus: 117th hour.

Variola Vaccinæ.

Calf No. 1 inoculated with small-pox virus: 144th hour.

Secondary vesicle.

Original sites of inoculation showing papules which were developed at the 117th hour fading.

Secondary vesicle

Secondary vesicles from which Calf No. 3 was inoculated—just before extraction of lymph. It is from this source that the present lymph stock of the Vaccine Supply Department was derived.

Vesicle destroyed by pressure in extracting lymph for Calf No. 2. This stock was lost on transfer from Calf No. 2 to a Buffaloe which proved immune.

CALF No. II.

24th March 1891.	100·4		.....	.....	.....	.....	13 punctures and two lines.
	48	102·	Faint blush.				
	72	101·2	Slightly raised, red blush more marked.				
	82	103·6	Punctures distinctly raised and papular; nothing distinguishable from ordinary cow-pox, except perhaps more general redness than usual at this hour.				
	96	101·	.....	Distinct pearly lines of vesicle formation with centres depressed, giving the appearance of umbilication.	.....	.....	Inoculated buffalo. [This inoculation failed; fresh ordinary animal lymph subsequently used, also failed.—W. G. K.]
	106	102·4	.....	.....	.....	Two papules, apparently secondary, found one near mamna and the other near the groin.	
	120	102·4	.....	.....	.....	Papules near the mamna and groin have developed into vesicles, with characteristic umbilications.	
30th March 1891.	144	101·2	.....	An abrasion caused during shaving of a linear shape was inoculated at the same time as other sites with a practically dry spatula, after apparent exhaustion of lymph. This now exhibits two continuous ridge-like vesicles. As regards the original size of punctures, which were all exhausted of lymph on the 28th March 1891, they are again filled with lymph. Round their central depressions, and within the area are small secondary papules, to the extent of 12.	.....	There are two more papules, apparently injured by moving the animal, on the perineum. The papules near the mamma and the left and right thighs have now developed into vesicles. They are symmetrical and round, and have deep umbilications and areolæ. On the right thigh are two fresh papules noticed.	

Statement showing successive grades of modification of small-pox virus undergone in becoming "vaccine"—contd.

Date.	Hour.	Temperature of animals.	Papule.	Vesicle.	Scab.	Secondary eruption.	Remarks.
31st March 189'.	168	101·	.....	The uninjured vesicles contain lymph but are losing their pearly aspect, and are now of a duller white.			
	192	101·2	.....	The vesicles are now distinctly flattened, and are approaching the usual scab stage.			
7th April 1891.	...	...	.....	Scabs loose and detached.			
8th April 1891.	...	100·6	.....	.....	All the scabs have fallen, with the exception of one on the left flank.		
15th April 1891.	...	...	.....	.....	.....	.....	Inoculated with ordinary fresh animal lymph, at 8 A.M. by 15 punctures.
18th April 1891.	72	...	Round the 15 punctures there was yesterday a slight redness and it was thought likely the vaccine would "take"; but it is now seen to have failed completely.				
				CALF No. III.			
26th March 1891.	...	102·2	.....	.....	.....	.....	This calf was inoculated from the *secondary* vesicles of calf No. 1 at 1 P.M.
27th March 1891.	28	101·4 102·6	Slight tinge of redness round each insertion.				

28th March 1891.	45 52	102·4 103·4	*Morning.* "Lines" are raised, punctures have assumed the papular form; red blush. *Evening:* no marked change.				
29th March 1891.	70	101·4 102·6	Papules are slightly more elevated than yesterday.	.....	.....	Between the two "lines," a papule of secondary origin has made its appearance.	Drawing made.
30th March 1891.	93	101 103·2	.....	All insertions are now distinctly vesicular, including the secondary papules, between the lines mentioned in yesterday's note. The lines now (at 12·30) appear fully developed. They are tense with lymph, and have the ordinary appearance of lines formed upon the abdomen of the calf during animal vaccine inoculation, except that they are full and raised to an extent rarely seen.	.....	There are on the left thigh six papules, four of which have a shotty feeling; no signs of redness about them. In the right groin there is one papule, and on the same thigh there are three. There is doubt whether these latter are caused by insect bites. They have not the shotty feeling as observed in the left thigh. Slight areolar redness around each puncture and lines.	Drawing made.
31st March 1891.	117	101·2 101 6	.....	.....	.....	On the left thigh the papules have still a shotty feeling. One has a whitish aspect, as if lymph was forming at its apex. On the right thigh only one papule is now noticeable. It has not the shotty feeling. The "secondary" vesicle between the lines is now fully developed. It has not the central depression. A papule is now noticed half an inch from this vesicle.	Last night lymph was taken for inoculation from calf No. IV from all vesicles and lines except the secondary one just described, and the four punctures; the latter is now full of lymph, and has a flattened appearance with central depression; has an areola extending about half an inch.

Statement showing successive grades of modification of small-pox virus undergone in becoming "vaccine"—contd.

Date.	Hour.	Temperature of animals.	Papule.	Vesicle.	Scab.	Secondary eruption.	Remarks.
5th April 1891.	238	101·4	.....	Vesicles are opaque and slightly flattened.	.....	Undergoing the process of scabbing.	Drawing made. Secondary papules have still a shotty feeling, and seem to have made no progress one way or the other; *i.e.*, as far as the left and right thighs are concerned. Suspicion arises that those on the thighs are from insect-bites, and the only secondary vesicle is that noticed between the lines, which is now drying up. [Subsequent experience would dictate that they were secondary papules, but that they aborted.—W. G. K.]
8th April 1891.	...	101·2	.....	....	.....	All scabs are formed.	

Note.—Inoculated this calf with fresh ordinary animal lymph on 17th April 1891, using ten insertions. All failed.

CALF No. IV.

30th March 1891.	At 6-30 P.M.	102·	.....	.....	.....	.....	This was inoculated at 6-30 from calf No. 135 III. There were made 93 punctures, 6 scarifications and 12 lines.
31st March 1891.	24	...	The site of punctures are slightly elevated and red, and present all general appearance of vaccine "taking."				

2nd April 1891.	39	101·M. 102·2E	.....	On the thin part of the skin in the groin, the papules are now assuming a vesicular form. The elevation is not great, and there is no inflammatory redness. The appearance is that of mild vaccine of 96 hours.			
3rd April 1891.	65	...	.....	.....	.....	.....	Transferred lymph to calf No. V.
4th April 1891.	108	...	.....	The vesicles are now well developed and tense with lymph. They have no areola or inflammation around them. The lines are broader than usually found in vaccine and not so elevated, but they are distinctly vesicular throughout their course.	.....	Suspicious papule is felt. This is now 108 hours after inoculation. It has a decidedly shotty feeling. There is also a large secondary papule on the abdomen, which was not noticed last night.	Last night the animal was exhibited to the Branch British Medical Association, Madras, when it was concluded that the animal was free of secondary eruption. Transferred lymph at 1 P.M. from vesicles becoming opaque to calf No. VI.
5th April 1891.	120	101·2	.....	The vesicles are now decidedly opaque, each surrounded with an areola about $\frac{1}{8}$ of an inch, now becoming more flattened.	.....	Doubt is now felt as to the vesicles spoken of as "secondary" being truly of that nature. On using a magnifying glass in the centre is found a slight elevation, as if caused by a puncture and there is no blood stain on it. A papule was found on the perineum yesterday. It has a shotty feeling.	An apparently glandular enlargement in the lumbar region about the size of a shot was observed. It has been noticed that calves inoculated up to date are markedly tender over the lumbar region on pressure.
7th April 1891.	160	101 6	.....	The vesicles are large and opaque; lines are broad and full but slightly flat tened; areola about $\frac{1}{8}$ inch.			

Statement showing successive grades of modification of small-pox virus undergone in becoming "vaccine"—contd.

Date.	Hour.	Temperature of animals.	Papule.	Vesicle.	Scab.	Secondary eruption.	Remarks.
8th April 1891.	184	102·6	.....	Opacity and commencing scabbing.	.....	Scabbing progressing.	
9th April 1891.	228	101·2	.....,	The process of drying is not yet complete. Each vesicle has an opaque appearance and is flattened—the size of each being remarkably great when compared with ordinary vaccine. Each vesicle formed from a puncture is not less than the area of a large "yanaigundumani," whilst the "lines" are so broad that in the process of drying they look as if caused by a red hot iron.			
12th April 1891.	...	...	.....	....	.....	Scabbing complete.	
				CALF No. V.			
3rd April 1891.	...	101·4	.....	.....	.....	.....	Inoculated from calf No. IV.
4th April 1891.	36	102·2	All lines and punctures have a slight tinge of redness.				
5th April 1891.	...	101·4	Every line and puncture has the usual slight redness surrounding them; as if all were "taking."	.....	.....	.....	Drawing made at 53rd hour.

6th April 1891.	74	101·2 101·2	Punctures and lines are little more raised, similar to vaccine at this hour.				
7th April 1891.	98	100·2	.....	Papules are large and very slightly flattened. Areola more marked, especially in the lines.	.....	.....	Drawing made at 98th hour.
8th April 1891.	122	103·4	.....	Vesicles and lines are large and full. The undisturbed vesicles are similar to those of vaccine at this hour.	.....	A small secondary vesicle is found by the side of a vesicle. It is small and accuminated.	Drawing made at 122nd hour. Paste was prepared from the vesicles and was retained as "stock" in case of failure.
9th April 1891.	146	102·	.....	Vesicles here bear no comparison as to size with those produced with the opaque lymph on calf No. VI. The same remark applies to the lines.			
10th April 1891.	192	102·4	.....	Vesicles are opaque	.....	.....	Drawing made at 192nd hour.
12th April 1891.	...	101·2	.....	.....	Scab complete & firmly adherent.		
16th April 1891.	...	...	.....	.....	Scabs fell from undisturbed vesicles.		
				CALF No. VI.			
4th April 1891.	...	...	.....	.....	.....	.....	Inoculated from calf No. VI at 1 P.M. The lymph from nearly all the vesicles was opaque. The transfer was made in consideration of the possibility of losing the stock, should calf No. V fail, and to ascertain also the result of employing "opaque" lymph.

Statement showing successive grades of modification of small-pox virus undergone in becoming "vaccine"—contd.

Date.	Hour.	Temperature of animals.	Papule.	Vesicle.	Scab.	Secondary eruption.	Remarks.
5th April 1891.	...	101· 101·6	Punctures and lines are all surrounded by a ring of redness and slight tumefaction. The latter being chiefly marked where small multiple scarifications had been carried out.				
6th April 1891.	44	101·	Punctures and lines still surrounded by redness.	.....	.....	.....	Drawing made at 44 hours.
8th April 1891.	...	102·4	.....	Vesicles are not quite full, lymph is free; no secondary eruption.			
9th April 1891.	115	103·	.....	Vesicles and lines are now full and large and have all the characteristic of fine vaccine vesicles in an over advanced condition.			
10th April 1891.	103·2	...	.....	Vesicles are large and flattened —the contents opaque.			
12th April 1891.	...	101·	.....	Subcutaneous inflammation and thickening at various parts over the abdomen.			
13th April 1891.	...	102·	.....	Vesicles are still not dry; they are very flattened and becoming dry in the centre. There is a good deal of subcutaneous inflammation.			

CALF No. VII.

6th April 1891.	...	103·2	....	....	.....	.....	calf No. VII was inoculated at 6 P.M. from Calf No. V.
8th April 1891.	...	102·4	Lines and punctures present redness as if "taking."				
9th April 1891.	62	...	Lines and punctures advancing in development.				
10th April 1891.	90	102·4	Presents the usual appearance of vaccine inoculation at this hour, but somewhat advanced: lymph flows freely on pressure with the forceps.	.....	.....	.....	At 12 noon inoculated calf No. VIII.
11th April 1891.	111	...	.....	Puncture and line sites are now almost fully developed as vesicles; but are much larger in size than those of ordinary vaccine; they are each surrounded by a limited areola. On the thick part of the skin of abdomen, the lines appear capable of further development without being overripe. Three lines on the thigh and on the thin part of the skin seem however to be fully filled with lymph. They have the true pearly appearance of vaccine vesicles. Along the lines there are slight borders of redness; but they are very narrow and are strictly limited.	.....	.....	At 108 hours, 3 grains of vesicle pulp and at 111 hours another 10 grains were collected; certain vesicles being left undisturbed to watch progress. One portion was kept to be used in the event of failure of stock, and another portion was sent to Mr. Gonsalvez for report. (See Report attached, page 68.)

Statement showing successive grades of modification of small-pox virus undergone in becoming "vaccine"—concld.

Date.	Hour.	Temperature of animals.	Papule.	Vesicle.	Scab.	Secondary eruption.	Remarks.
12th April 1891.	...	101	.....	Undisturbed vesicles still larger and opaque; slightly depressed still yield lymph when squeezed.			
13th April 1891.	...	101	.....	Vesicles becoming flattened; areola around each is small, but there is evident tumescence around each. This is especially marked where the "lines" have been placed close to each other: where the "lines" have been removed for making paste, there is almost a complete absence of tumescence, whilst it is especially marked when they have been allowed to remain.			
				CALF No. VIII.			
10th April 1891.	...	101·4	.....	.....	.....	.....	Inoculated at 12 noon from calf No. VII.
12th April 1891.	...	101·2	Is taking slowly.				
13th April 1891.	...	101·	Punctures exhibit papular elevation, but these are small. The "lines" are undergoing similar elevation.	.....	.....	.....	There is a glandular enlargement in the groin of the left thigh.

14th April 1891.	101 hours.	...	.....	Vesicles full.	.....	.....	Took 15 grains of pulp at 5 P.M. With this paste vaccinated children at Ooty and started the Bangalore Depôt with the same paste for "stock."
15th April 1891.	116 hours.	...	.....	The undisturbed vesicles are now decidedly smaller than at a similar stage in former generations.	.....	There is no appearance of any secondary eruption.	The glandular enlargement has disappeared.
16th April 1891.	140 hours.	100·	.....	The undisturbed vesicles are now larger and full, but one is slightly opaque. There is practically no inflammation, and the areola round undisturbed vesicles is not more than one or two lines in breadth.			

Statement of the progress of the two first cases of vaccination of the human being with small-pox virus modified by transmission through bovines, reported by Assistant Surgeon Gonsalvez, Acting District Medical and Sanitary Officer, Anantapur.

Name, Narrainappa ; *Age,* 6 months ; *No. of scarifications,* 4 ; *No. of vesicles,* 4 ; *Date of vaccination,* 15th April 1891.

In this case four scarifications were made. For the first three days the scarified part appeared red, but on the 4th day the parts became elevated. On the 5th day the elevation became more marked, and on the evening of the same vesicles were seen. On the 7th day, vesicles fully developed, centre depressed. On the 8th day, vesicles large and contained clear transparent lymph, areola forming. On the 10th day, vesicles changed into pustules and areola disappeared. On the 14th day, scab began to form. A chart, indicating temperature, is herewith attached.

Name, Hanumantha Rau ; *Age,* 6 months ; *No. of scarifications,* 4 ; *No. of vesicles,* 4 ; *Date of vaccination,* 15th April 1891.

Four scarifications, two on each arm were made, which for the first two days appeared red. On the 3rd day these were elevated, which on the 4th day became more marked. On the 5th day, minute vesicles were formed ; these on the 7th day developed into full vesicles distended with clear virus, and the centre umbilicated. On the 8th day, the vesicles were seen more developed, and areola well marked. On the 10th day, a black spot appeared in the centre, but the areola was still marked. On the 14th day, scabs began to form and the areola disappeared. A chart, indicating temperature of the child vaccinated during the course, is herewith attached.

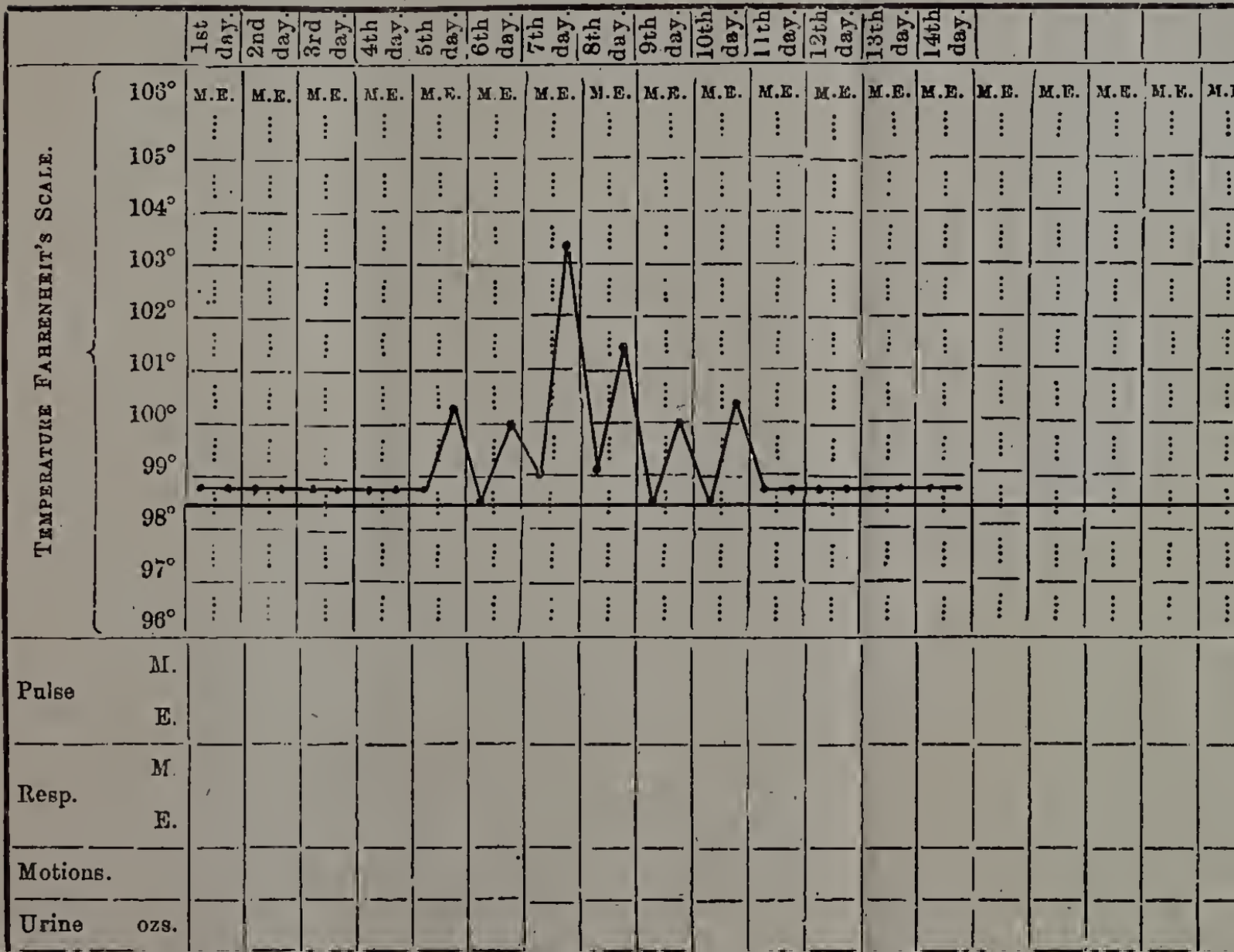
CLINICAL CHART OF TEMPERATURE, &c.

Name Narrainappa, *Age* 6 months, Vaccinated on 15th April 1891, *Result* Successful.

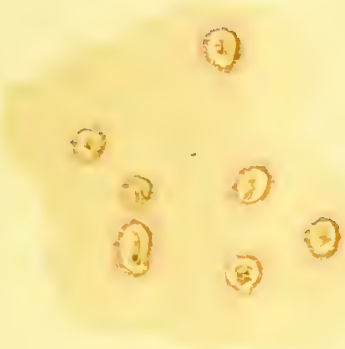
	1st day.	2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	11th day.	12th day.	13th day.	14th day.							
Temperature Fahrenheit's Scale. 106°	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	M.E.	41° Temperature Centigrade Scale.
105°																					
104°																					40°
103°																					
102°																					39°
101°																					
100°																					38°
99°																					
98°																					37°
97°																					
96°																					36°
Pulse M.																					Pulse M.
Pulse E.																					Pulse E.
Resp. M.																					Resp. M.
Resp. E.																					Resp. E.
Motions.																					Motions.
Urine ozs.																					Urine ozs.
,, Sp. Gr.																					,, Sp. Gr.
,, Reaction.																					,, Reaction.
,, Chlorides.																					,, Chlorides.
,, Albumen.																					,, Albumen.
Day of Dis.																					Day of Dis.

CLINICAL CHART OF TEMPERATURE, &c.

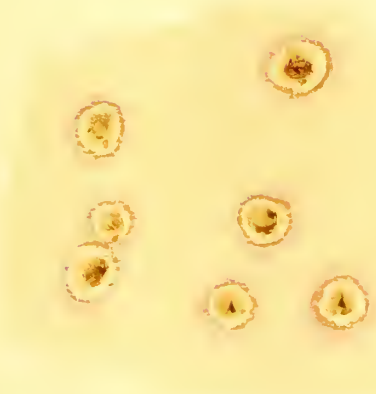
Name Hanumantha Rau, *Age* 6 months, Vaccinated on 15th April 1891, *Re*



116 hrs.



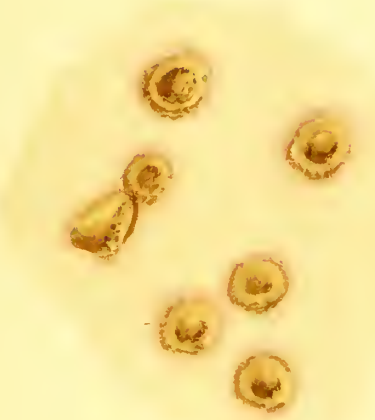
140 hrs.



The development of vesicles in the eighth generation from small-pox virus in the calf—actual size.

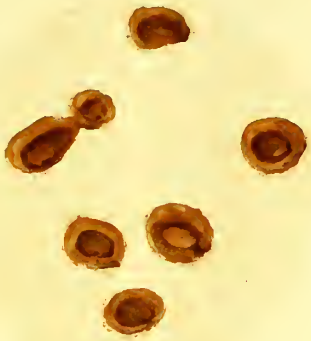
164 hrs.

188 hrs.



The development of vesicles in the eighth generation from small-pox virus in the calf—actual size.

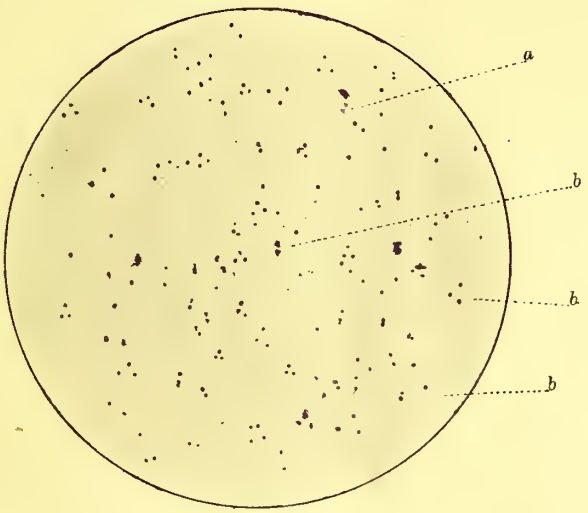
236 hrs.



The development of vesicles in the eighth generation from small-pox virus in the calf—actual size.

The development of vaccine vesicles on the calf from the 24th to the 120th hour by the "line" method of inoculation.

PLATE IV.



(AFTER BUIST.)

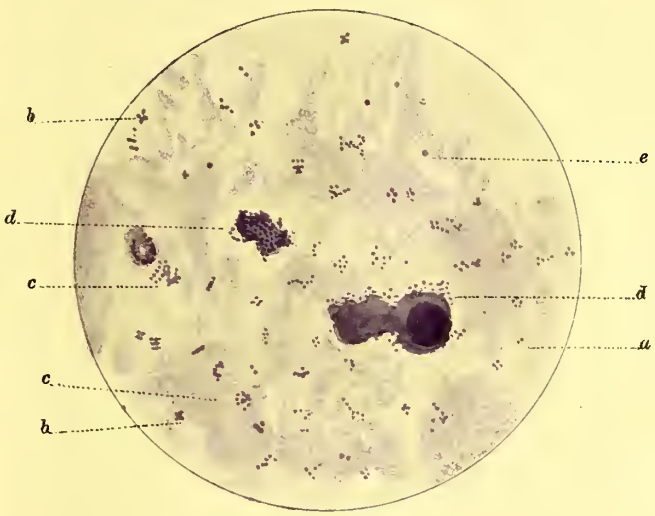
CLEAR VACCINE LYMPH.

SOURCE, Typical Jennerian vesicle. Cover-glass preparation.

a Minute isolated spores of micrococci.

b b b Larger forms of micrococci, showing commencement of opacity. Size of units varies from .1 μ to .5 μ.

PLATE V.



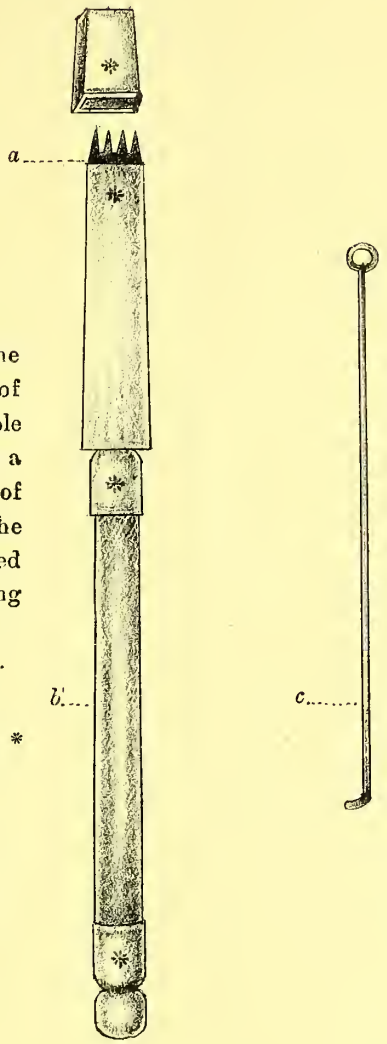
(AFTER BUIST.)

OPAQUE VACCINE LYMPH.

SOURCE, Preparation by Dr. Francis Troup, *from lymph in capillary tube.* This preparation shows:—*a* Diplococci. *b b* Sarcina-form groups. *c c* Chains or necklaces. *d* Irregular colonies or clumps. *e* Very large torula-form micrococci, like oil-drops when unstained. The size of the organisms varies from .5 μ to 2 μ. Koch's aniline methyl-violet stain.

Zeiss, Obj. K. Water immersion. Oc. 3. Magnification. 1045 diameters.

PLATE VI.



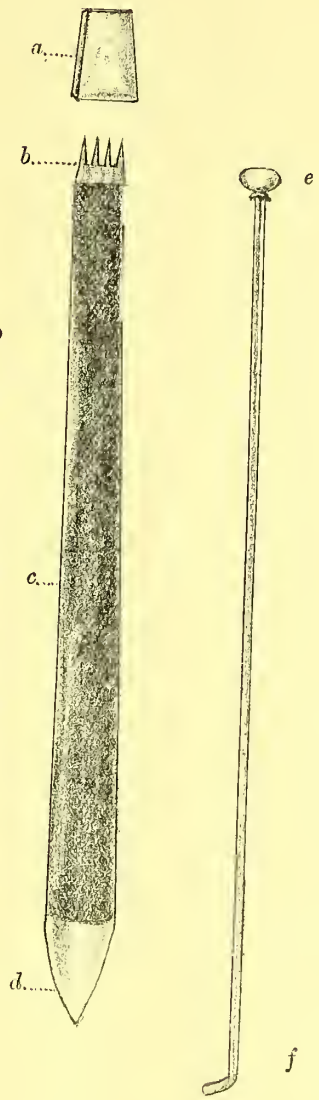
(*a*) The rake. The extent of protrusion of the "rake" is capable of being regulated by a screw in the body of the instrument. The "rake" can be removed entirely for cleansing or renewal.
(*b*) Vulcanite handle.
(*c*) Silver measure.

Parts marked thus * are electro-plated.

DR. KING'S SCARIFICATOR WITH PASTE MEASURE.

PATTERN No. 1.

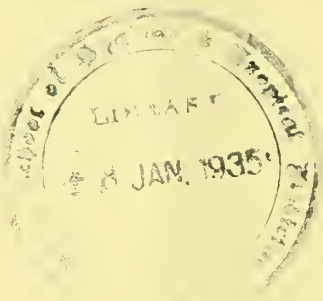
PLATE VII.

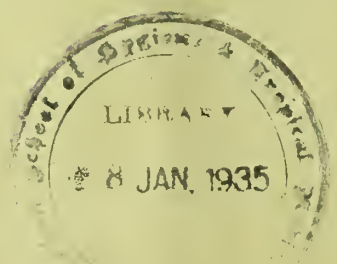


(*a*) Electro-plated cap for protecting points.
(*b*) Burnished steel points.
(*c*) "Blued" steel handle.
(*d*) Spatula end for removing paste from measure, of burnished steel.
(*e*) Flattened knob for guiding the paste into the scratches made by scarificator.
(*f*) Paste measure.

DR. KING'S SCARIFICATOR WITH PASTE MEASURE.

PATTERN No. 2.


JAN. 1935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8 JAN. 1935